

Table 1. Pollinarium and pollinium characters of Periplocoideae, Secamonoideae, *Fockea*, and Asclepiadoideae (excl. *Fockea*). The definition of pollinarium is taken from Endress (1994: 472) as the “apparatus for the transport of pollinia (the pollinia included).” In Asclepiadoideae (excl. *Fockea*), the characters “exine” and “distal walls” refer to the pollinium wall.

	Periplocoideae	Secamonoideae	<i>Fockea</i>	Asclepiadoideae (excl. <i>Fockea</i>)
Manner of attachment to pollinator	Viscidium (adhesive disc)	Corpusculum	Corpusculum	Corpusculum
Number of pollinia per anther	4 (loose pollinia)	4	2	2
Pollinium wall	Absent	Absent	Absent	Present
Composition of pollinium	Tetrads	Tetrads	Tetrads	Single grains
Exine	2-layered (tectum and granular stratum)	3-layered (tectum, well-developed granular layer and foot layer)	3-layered (tectum, thin granular layer and foot layer)	3-layered (tectum, thin granular layer and foot layer)
Distal walls (exine and intine)	3-layered	4-layered	4-layered	4-layered
Proximal walls (exine and intine)	3-layered	2-layered	2-layered	Not applicable

(1994, 1995) provided an overview of pollinium and/or pollinarium structure in the family. The purpose of this study is to provide information concerning the pollinium and pollinium wall structure of the three subfamilies according to our own observations, and is not meant to be a comprehensive palynological study.

MATERIAL AND METHODS

Tetrads and pollinia were obtained from herbarium specimens and fresh material collected in the field. Herbaria from which material was obtained are BLFU, BM, BR, K, L, MO, NH, P, PRE, SRGH, WAG, and WIND. Specimens investigated are indicated in Appendix 1.

Material collected in the field was fixed and flowers obtained from herbarium specimens were rehydrated in 3% phosphate-buffered glutaraldehyde. For scanning electron microscopy (SEM) pollinaria were removed from flowers in 100% ethanol. The pollinaria were air dried and mounted on stubs using double-sided tape, coated with gold, and examined with a Jeol Winsem 6400 microscope at 5 kV. For transmission electron microscopy (TEM) the pollinia were postfixed in 2% osmium tetroxide, stained with 0.5% uranyl acetate, dehydrated in an alcohol series, and embedded in Spurr's low-viscosity resin. Sections were stained with uranyl acetate, followed by lead citrate, and examined with a Philips CM 100 electron microscope at 60 kV.

Pollinium morphology is as described by Verhoeven and Venter (1994a). A pollinium is the con-

tents of one microsporangium (pollen sac), forming one pollen-unit. Endress (1994: 472) defined pollinarium as the “apparatus for the transport of pollinia (the pollinia included) as occurring in Asclepiadaceae and Orchidaceae.” A massula is considered to be a pollen unit larger than a polyad, but not the entire product of one microsporangium (pollen sac). Accordingly, each anther in the Periplocoideae produces four free pollinia, not massulae, as was reported by Nilsson et al. (1993). The terminology used for the walls of the pollinium and tetrads (Verhoeven & Venter, 1998a) is as follows: The distal walls are the tetrad walls that face toward the outside (circumference) of the pollinium. The proximal walls are the walls that separate tetrads on the inside of the pollinium. The inner walls are the walls that separate individual pollen grains of a tetrad. See also Table 1 for additional details of pollinaria and pollinia characters.

RESULTS

PERIPLOCOIDEAE

The Periplocoideae are characterized by having tetrads or free pollinia, which are shed onto cone-shaped or spoon-like translators, each of which consists of an adhesive disc (by which the translator sticks to the pollinator), a stalk and an adhesive-lined receptacle (onto which the tetrads or pollinium is shed at anthesis), as exemplified by *Finlaysonia* Wall. (Fig. 1A). The five translators present in a *Raphionacme* Harv. flower are positioned be-

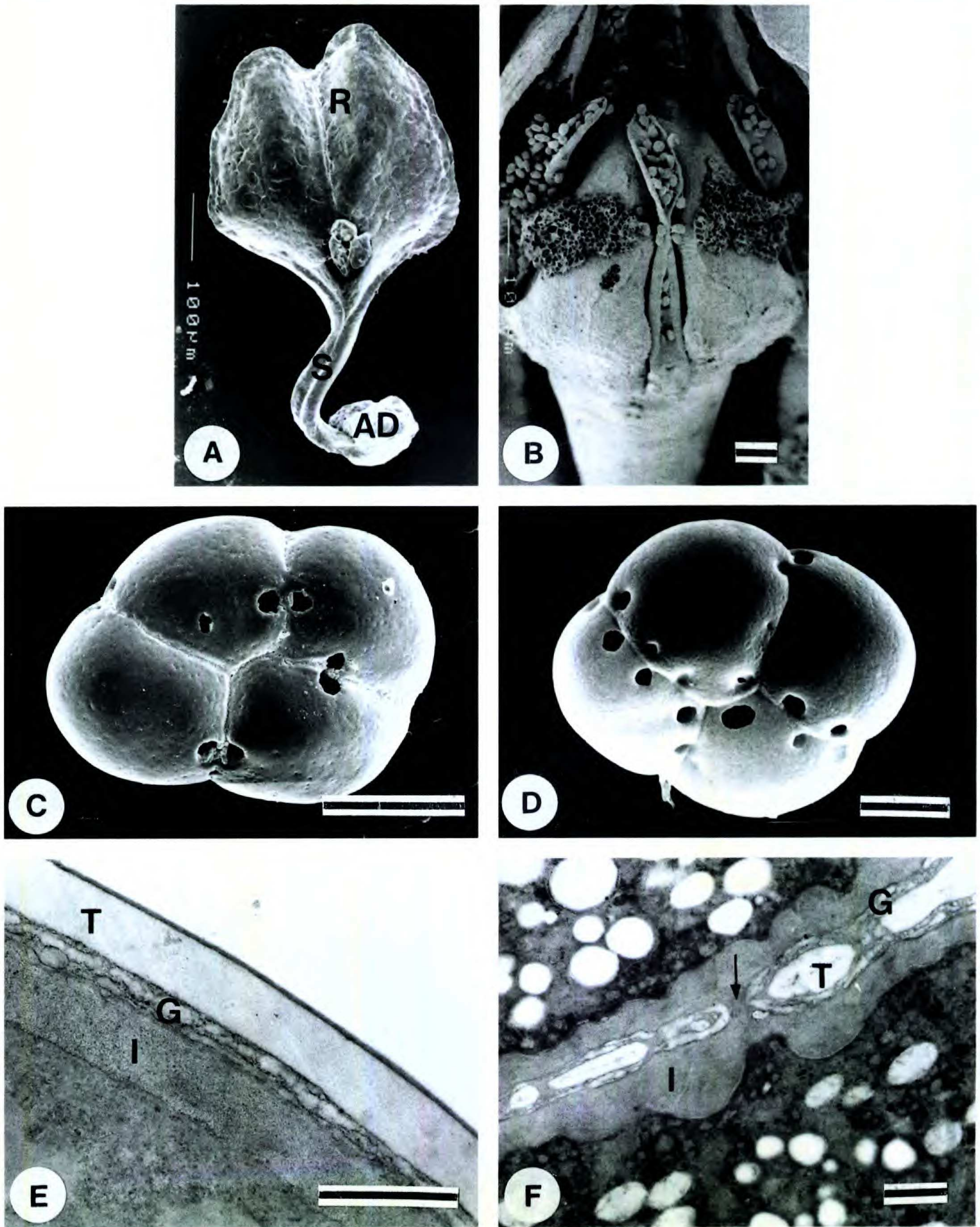


Figure 1. A. *Finlaysonia khasiana*. Translator showing adhesive disc (AD), stalk (S), and receptacle (R). —B. *Raphionacme hirsuta*. Translator positioned between anthers (removed) in a groove on stigmatic head. —C. *Cryptolepis albicans*. Rhomboidal tetrad. —D. *Raphionacme madiensis*. Rhomboidal tetrad. —E. *Raphionacme hirsuta*. Section of wall showing tectum (T), granular stratum (G), and intine (I). —F. *Raphionacme palustris*. Inner wall showing wall bridge (arrow), tectum (T), granular stratum (G), and intine (I). Scale bars: A & B = 100 µm; C = 20 µm; D = 10 µm; E & F = 1 µm.

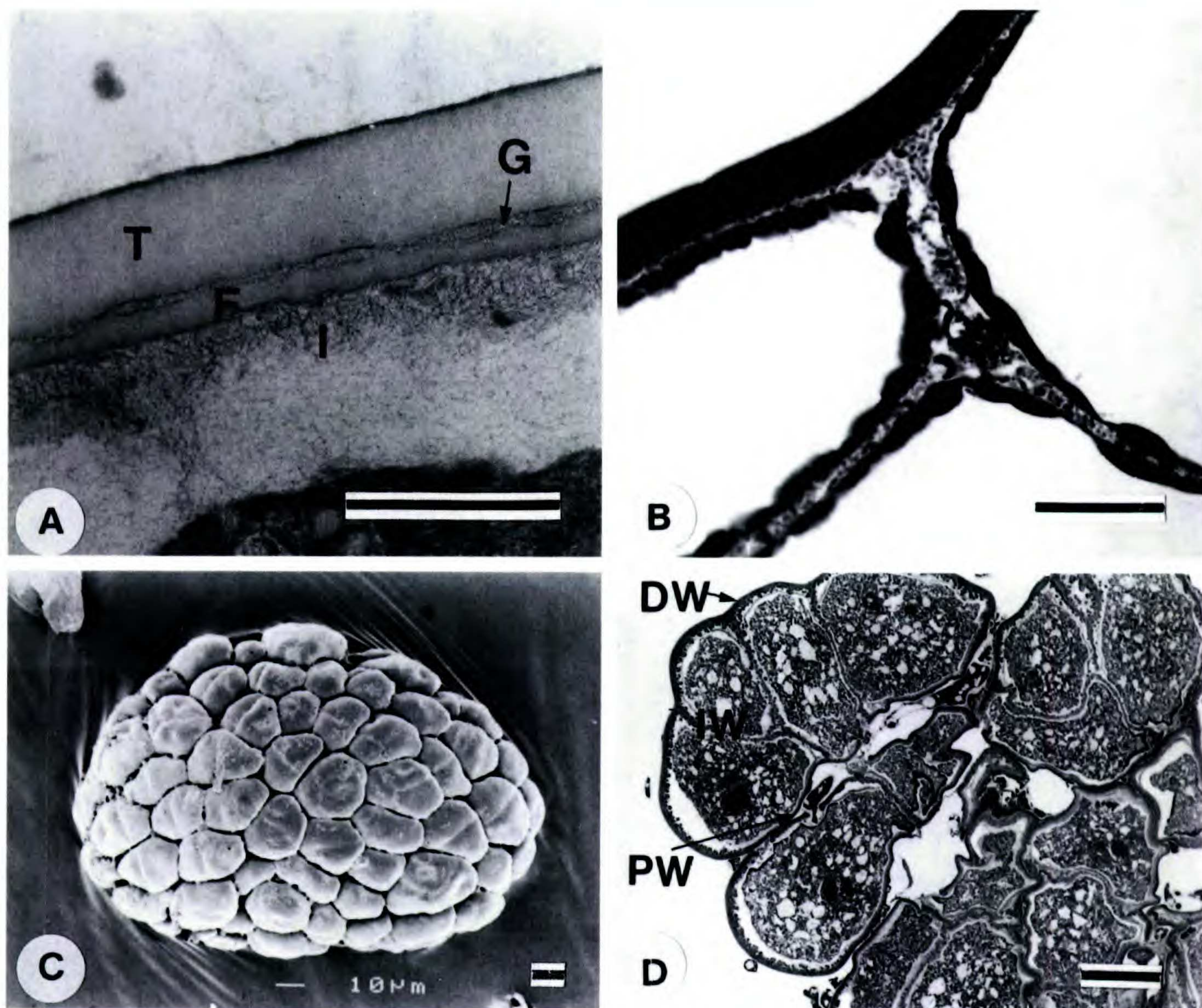


Figure 2. A, B. *Camptocarpus linearis*. —A. Section of wall showing tectum (T), granular stratum (G), foot layer (F), and intine (I). —B. Section of acetolyzed tetrad showing outer and inner wall of tetrad. C, D. *Hemidesmus indicus*. —C. Pollinium. —D. Section of pollinium showing tetrads, distal (DW), proximal (PW), and inner wall (IW). Scale bars: A = 0.5 μm ; B = 1 μm ; C & D = 10 μm .

tween the anthers, with the stalk fitting in a groove on the stigmatic head (Fig. 1B). Translator, tetrad, and pollinium morphology of Periplocoideae have been described by Schick (1982), Kunze (1993), Nilsson et al. (1993), Verhoeven et al. (1989), and Verhoeven and Venter (1988, 1993, 1994a, b, 1997, 1998a, b). The tetrad and pollinium morphology of the Periplocoideae will be discussed very briefly for the purpose of this paper (see also Table 1). The calymmate tetrads are generally arranged rhomboidally or decussately; however, tetragonal, tetrahedral, linear, and T-shaped arrangements are also present in some genera. The number of pores varies from 4 to 6 in most genera, such as *Cryptolepis* R. Br. (Fig. 1C). *Raphionacme*, however, has 8 to 16 pores per pollen grain (Fig. 1D). In all the genera the exine is smooth and consists of a distal stratum (tectum) subtended by a granular stratum (Fig. 1E) as seen in *R. hirsuta* (E. Mey.) R.

A. Dyer. The inner walls separating the individual grains of the tetrad have the same structure as the exterior wall, consisting of tectum, granular stratum, and intine. The walls are, however, not continuous, but interrupted by wall bridges consisting of intine and a granular stratum (Fig. 1F) as seen in *Raphionacme palustris* Venter & R. L. Verh. *Camptocarpus* Decne. (Fig. 2A, B) differs from other Periplocoideae genera in that the exine consists of a tectum, granular stratum, and foot layer. The inner walls consist of a granular layer, which on both sides is subtended by a foot layer and intine.

Seven genera of Periplocoideae have pollen coherent into pollinia, rather than tetrads, at anthesis. They are *Decalepis* Wight & Arn., *Epistemma* D. V. Field & J. B. Hall, *Finlaysonia* Wall., *Gymnanthera* R. Br., *Hemidesmus* R. Br., *Schlechterella* K. Schum., and *Streptocaulon* Wight & Arn. In taxa with pollinia, such as *Hemidesmus indicus* (L.)

Schult., each anther produces four free pollinia (Fig. 2C). The pollinia, while in the anther, are not attached to a translator. However, by anthesis the pollinia are shed onto the adhesive-lined receptacle of the translator. The pollinium is not covered by a pollinium wall; it consists of loosely coherent, but not fused, tetrads (Fig. 2D, Table 1). The distal wall exine is smooth and consists of an outer compact stratum (tectum), subtended by a granular stratum. In all genera except *Schlechterella*, the tetrads forming a pollinium exhibit a marked reduction in the number of pores in the distal wall (Fig. 2C; see also numerous SEMs and TEMs in Verhoeven & Venter, 1998a, 1998b). The proximal wall has the same exine stratification as the distal wall. The proximal wall is, however, thinner than the distal wall and pores are also present (Verhoeven & Venter, 1998a). The inner wall, with wall bridges, consists of a tectum, granular stratum, and intine. The exception is *Finlaysonia* Wall., where the inner wall only consists of a granular stratum and intine (Verhoeven & Venter, 1998a).

SECAMONOIDEAE

The Secamonoideae are characterized by having five pollinaria, each of which consists of four pollinia attached to the translator. In *Pervillaea venenata* (Baill.) Klack. the four pollinia are sessile on the translator, and in *Secamone gerrardii* Harv. ex Benth. the four pollinia are attached to a single rather indistinct caudicle (or dorsal process, Kunze, 1993) (Fig. 3A, B). The manner in which the pollinia are attached to the translator is especially variable in this subfamily, and is covered in detail in Civeyrel (1996; Civeyrel & Rowe, 2001 this volume). The pollinium consists of calymmate tetrads, which are coherent but not fused, and is not surrounded by a pollinium wall (Fig. 3C, Table 1). The distal tetrad wall consists of three layers: an outer tectum, well-developed granular layer, and a foot layer subtended by an intine (Fig. 3D). Pores were not observed in either distal or proximal walls. The proximal walls between tetrads consist only of a granular exine layer subtended by an intine (Fig. 3E). The inner walls, separating individual grains of a tetrad from each other, consist of a granular exine layer subtended by an intine (Fig. 3F). The granular exine layer is not continuous but is interrupted by intine bridges (Fig. 3F, arrow).

ASCLEPIADOIDEAE

In the Asclepiadoideae each of the five anthers produces two pollinia, which develop from the two ventral (fertile) pollen sacs. The Asclepiadoideae

are thus characterized by having five pollinaria, each of which consists of two pollinia, each attached to the corpusculum by a caudicle (Fig. 4A–D). The pollinium consists of single, inaperturate pollen grains and is surrounded by a pollinium wall (Fig. 4E, F). The pollinia show a great variation in form, varying from oblique-obovate in *Asclepias curassavica* L. (Fig. 4A), hemispherical in *Mateleia brevicoronata* (B. L. Rob.) Woodson (Fig. 4B), oblong in *Microloma sagittatum* (L.) R. Br. (Fig. 4C) to reniform in *Stapelia gigantea* N. E. Br. (Fig. 4D). In *Stapelia gigantea*, an extrapollinial appendage, called a wing (El-Gazzar & Hamza, 1973), is present along one side of the pollinium (Fig. 4D). The genus *Fockea* Endl. (Fockeeae), however, differs from the other tribes in that the pollinium consists of calymmate tetrads, which are coherent, but not fused, and is not surrounded by a pollinium wall as shown in *F. angustifolia* K. Schum. and *F. sinuata* (E. Mey.) Druce (Fig. 5A, B, Table 1). The translator consists of a corpusculum, which is clearly divided by a longitudinal cleft. On the ventral part of the corpusculum adhesive material is present, which spreads out to form two lateral adhesive pads (Fig. 5A). The two pollinia are each attached to the upper region of the corpusculum by a short obscure caudicle (or dorsal process, Kunze, 1993). The wall structure of the tetrads forming the pollinium differs as to the distal and proximal walls. The distal wall consists of a tectum (distal exine), thin granular layer, foot layer, and intine as seen in *F. multiflora* K. Schum. (Fig. 5C). The proximal walls between individual tetrads are separated by a space and consist only of a granular exine layer subtended by an intine (Fig. 5D). The inner walls, separating individual grains of a tetrad from each other, consist of a granular exine layer subtended by an intine. The granular layer is not continuous but is interrupted by intine bridges (Fig. 5D). No pores were observed in either distal or proximal walls.

The tribes Marsdenieae, Ceropegieae, and Asclepiadeae in Asclepiadoideae all have a similar pollinium wall structure. The pollinium wall as in *Cynanchum ellipticum* (Harv.) R. A. Dyer consists of a tectum (distal exine layer), a thin granular layer, a foot layer, and intine (Fig. 6A). The tectum (distal exine layer), granular layer, and foot layer together form the ectexine. The granular layer varies from well developed in *Ceropegia stapeliiformis* Haw. (Fig. 6B), to weakly developed in *Gymnema sylvestre* (Retz.) R. Br. ex Schultes (Fig. 6C), where it is present as a discontinuous line. In Asclepiadoideae examined the tectum (distal exine layer) is usually thicker than the foot layer (Fig. 6A). In *Stapelia* L. and *Ceropegia* L. (Ceropegieae)

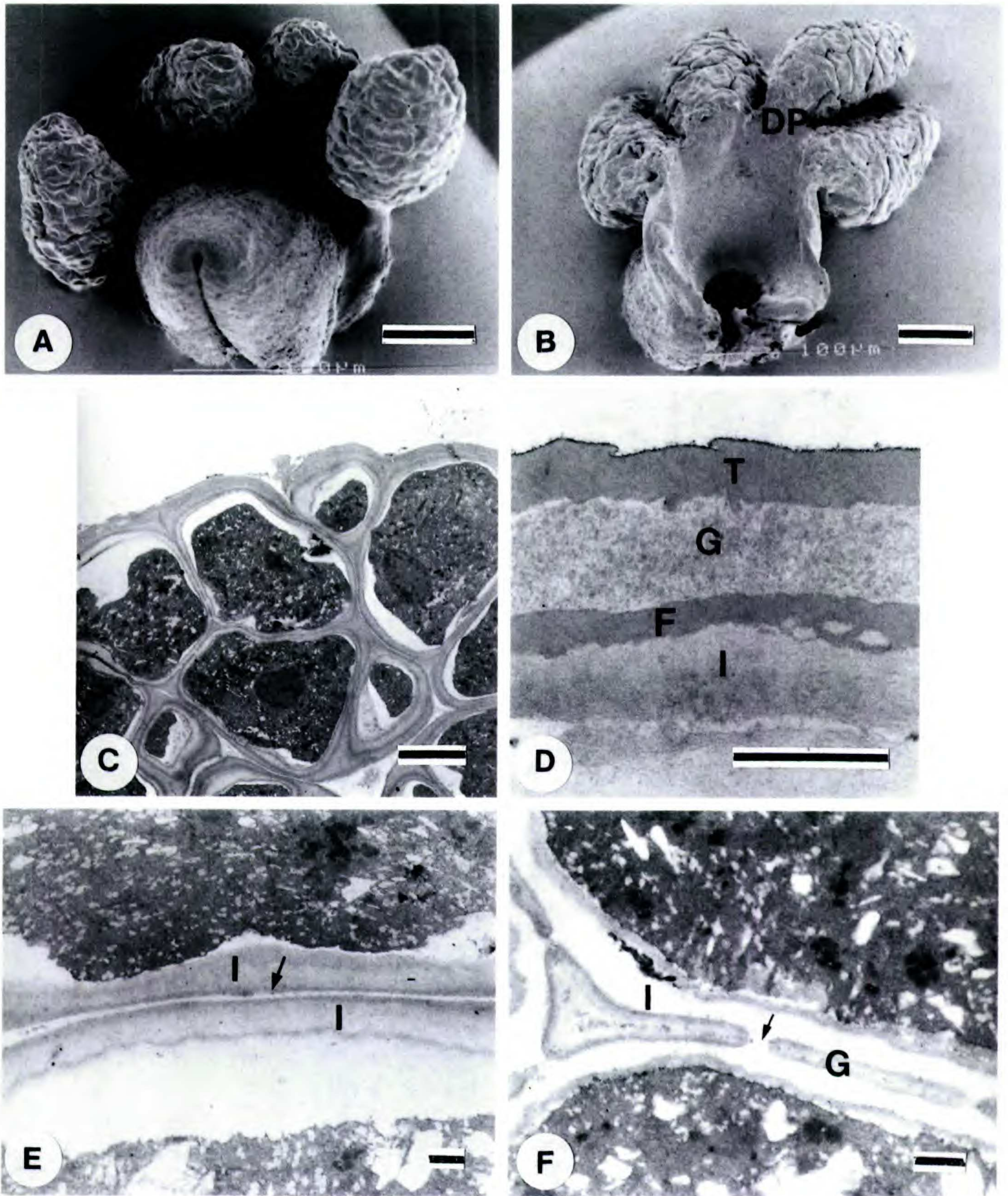


Figure 3. *Secamone gerrardii*. —A. Pollinarium with four pollinia. —B. Pollinarium showing indistinct caudicle (dorsal process) (DP). —C. Section of part of pollinium showing tetrads. —D. Distal tetrad wall showing tectum (T), granular infratectum (G), foot layer (F), and intine (I). —E. Proximal walls between tetrads showing granular exine layer (arrow) subtended by intine (I). —F. Inner wall consisting of granular exine layer (G), intine (I), and wall bridges (arrow). Scale bars: A & B = 100 μm ; C–F = 1 μm .

the tectum (distal exine layer) is thinner than the foot layer (Fig. 6B, D). The inner walls separating individual pollen grains are characterized by a granular layer, which forms the separation layer. On each side of the granular layer a foot layer and intine are present as shown here by *Stapelia gi-*

gantea and *Matelea reticulata* (Engelm. ex A. Gray) Woodson (Fig. 6D, E). *Tylophora flanaganii* Schltr. (tribe Asclepiadeae) differs from the other genera investigated. Its pollinium wall consists of columellae and a very thin foot layer, which is subtended by intine (Fig. 6F).

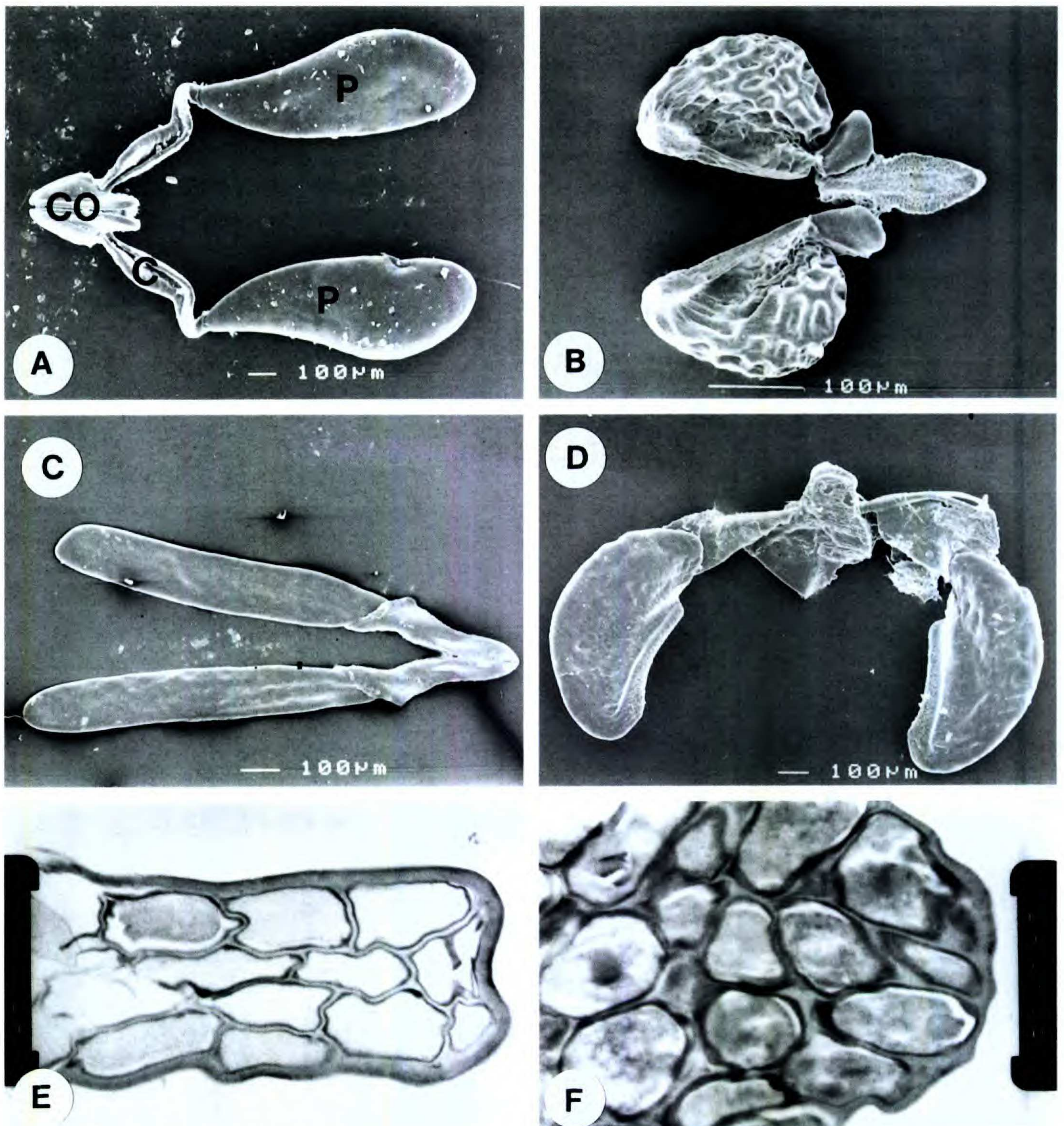


Figure 4. A–D. Pollinaria. —A. *Asclepias curassavica*. —B. *Matelea brevicoronata*. —C. *Microlooma sagittatum*. —D. *Stapelia gigantea*. E, F. Section of pollinia showing single pollen grains. —E. *Xysmalobium undulatum*. —F. *Cynanchum ellipticum*. C—caudicle, CO—corpusculum, P—pollinium. Scale bars: A–E = 100 μm ; F = 66.6 μm .

DISCUSSION

Tetrad morphology is very similar throughout the Periplocoideae. *Raphionacme* is the only genus that can be distinguished by its multiporate pollen grains in contrast to only 4 to 6 pores in the other genera. Tetrads of *Raphionacme*, having numerous pores, could be regarded as more advanced than genera with 4 to 6 pores (Nilsson et al., 1993), but a detailed phylogenetic analysis would need to be carried out in order to determine this. The exine structure, consisting of a solid stratum (tectum)

subtended by a granular stratum, varies little within the Periplocoideae tetrads and pollinia. The inner walls, which separate individual pollen grains of a tetrad, have the same structure as the exterior wall. The walls are, however, thinner and more irregular in appearance than the outer walls. In *Camptocarpus* the structure of the walls differs from the other genera in that the exine consists of a tectum, granular stratum, and foot layer (Verhoeven & Venter, 1994a). The inner walls also differ from the general structure in that the tectum is absent. This inner

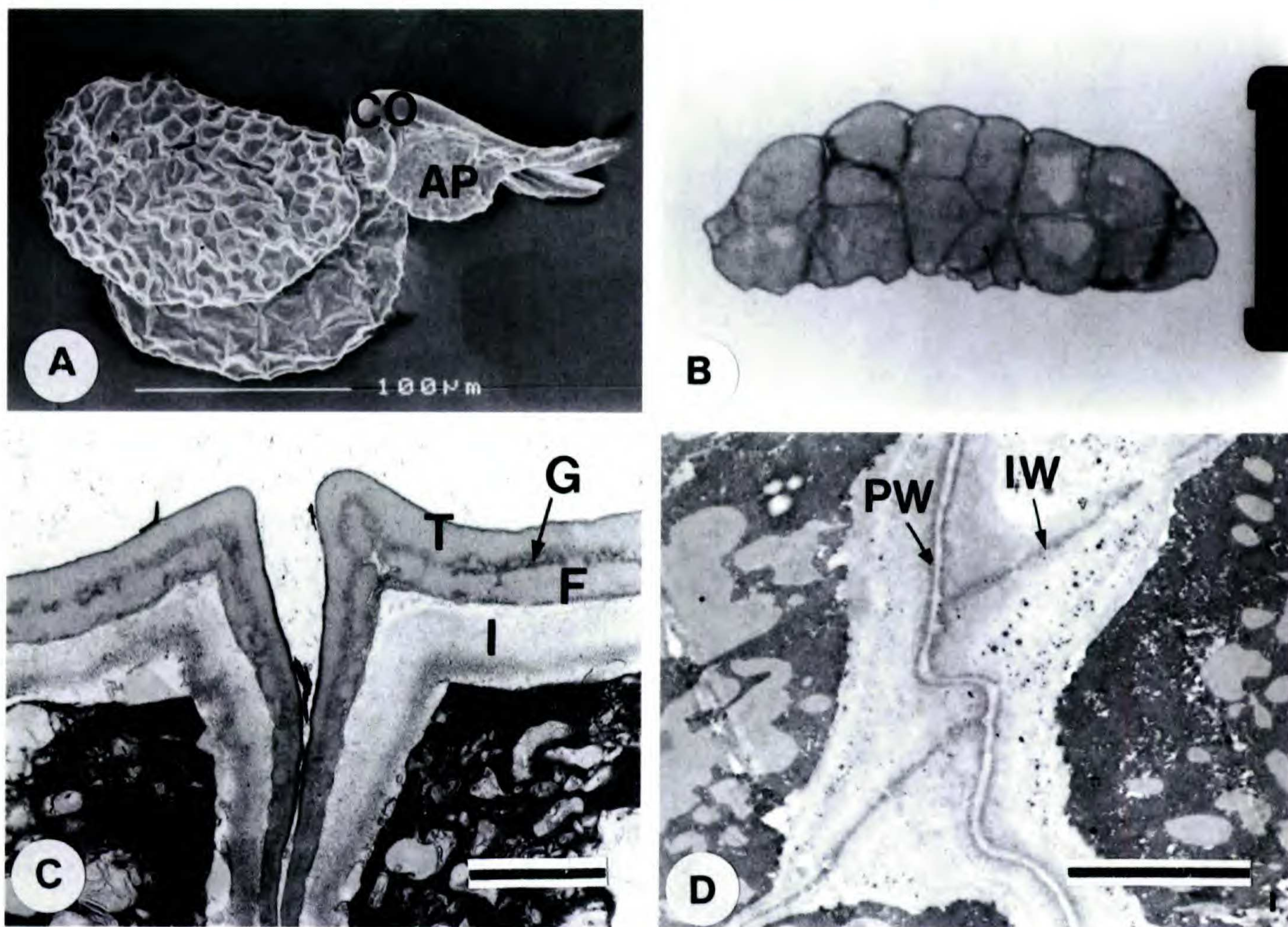


Figure 5. *Fockea*. —A. *F. angustifolia*. Pollinarium showing corpusculum (CO) and adhesive pads (AP). —B. *F. sinuata*. Section of pollinium showing tetrads. —C. *F. multiflora*. Two adjoining tetrads with distal walls showing tectum (T), granular layer (G), foot layer (F), and intine (I). —D. *F. sinuata*. Proximal walls (PW) between tetrads and inner walls (IW). Scale bars: A & B = 100 μm ; C = 1 μm ; D = 5 μm .

wall, consisting of a granular layer, which on both sides is subtended by a foot layer and intine, is similar to the walls surrounding the individual pollen grains in Asclepiadoideae pollinia. While this would suggest that *Camptocarpus* is palynologically more advanced than the other taxa of Periplocoideae, its floral morphology is otherwise unremarkable (Venter & Verhoeven, 1997; Klackenberg, 1998), as is its position in molecular studies (Civeyrel et al., 1998).

An interesting correlation, which has not received due attention to date, is the reduction in the number of pores in the distal walls, and the presence of pollinia. Whereas in Periplocoideae taxa with pollen as loose tetrads the average number of pores per pollen grain is 4 to 6, in the Asian (and Australian) genera in which the tetrads are coherent into pollinia, the distal wall of the pollen tetrads is inaperturate or only vestigially porate (Civeyrel, 1996). The only genera of Periplocoideae from Africa with pollen tetrads coherent into pollinia are *Schlechterella* K. Schum. and *Epistemma* D. V. Field & J. B. Hall. In contrast to all other pollinia-bearing Periplocoideae, in *Schlechterella* the tetrads

that make up a pollinium are multi-porate. The only other genus of Periplocoideae with multi-porate tetrads is *Raphionacme*, suggesting a relationship between these two genera. Indeed *Schlechterella abyssinica* (Chiov.) Venter & R. L. Verh. was previously included in the genus *Raphionacme* (Verhoeven & Venter, 1988) or sometimes as a separate genus *Triodoglossum* Bullock, which is a newer synonym of *Schlechterella*. Results of molecular analyses also suggest a close relationship between *Schlechterella* and *Raphionacme* (Civeyrel et al., 1998). The presence of pollinia composed of multi-porate tetrads in *Schlechterella*, as opposed to nearly inaperturate tetrads in other genera, suggests that pollinia may have arisen twice within Periplocoideae.

Pollinia in Periplocoideae and Secamonoideae are similar in that the pollinium consists of tetrads and is not covered by a pollinium wall, as it is in the Asclepiadoideae (Table 1). However, the wall structure of the tetrads forming the pollinium differs in the two subfamilies. In the Periplocoideae the wall consists of a tectum, granular stratum, and intine. The structures of the distal and proximal walls are morphologically similar; however, the

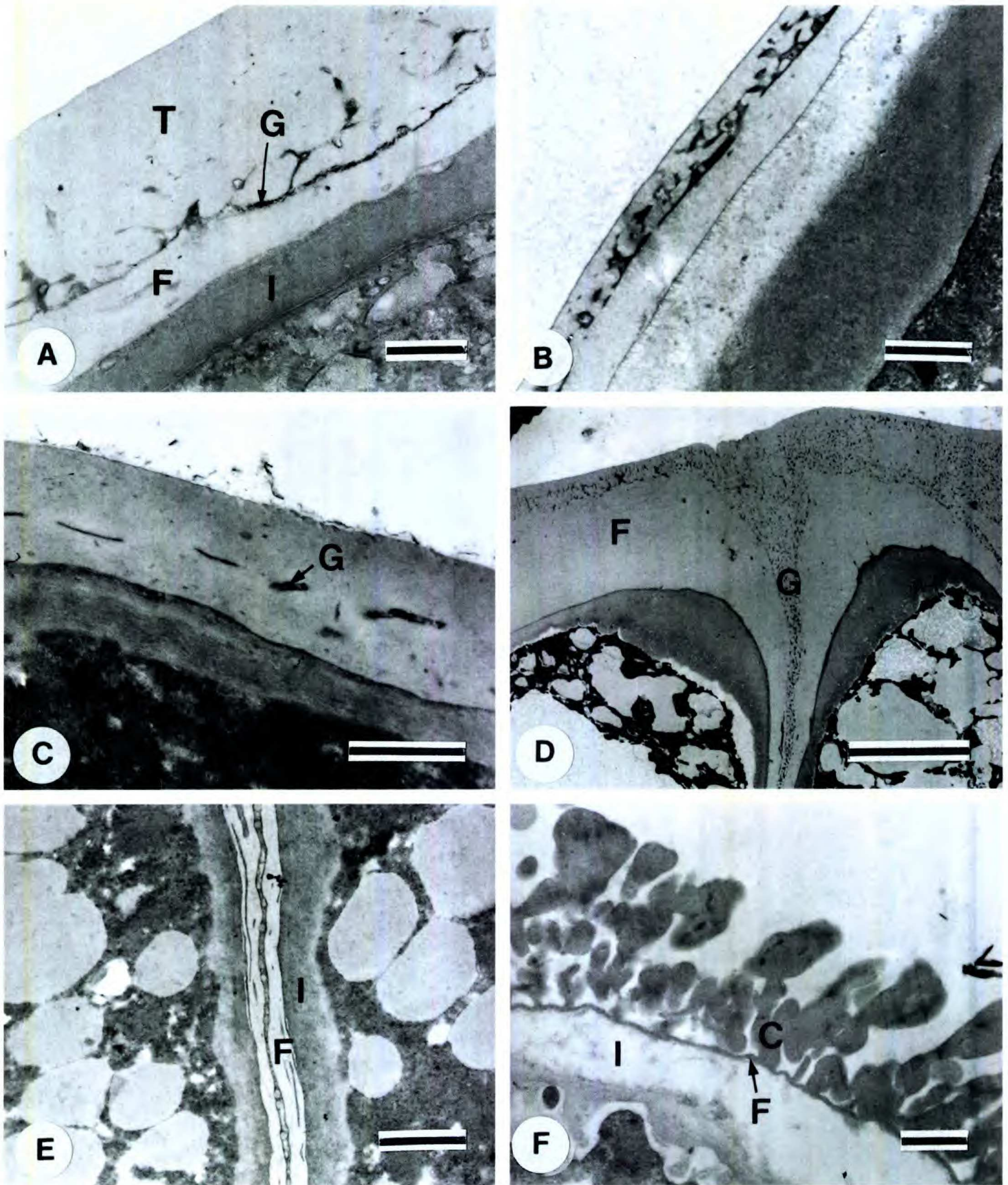


Figure 6. —A. *Cynanchum ellipticum*. Pollinium wall showing tectum (distal exine layer) (T), granular layer (G), foot layer (F), and intine (I). —B. *Ceropogia stapeliiformis*. Pollinium wall showing well-developed granular layer. —C. *Gymnema sylvestri*. Pollinium wall showing weakly developed granular layer (G). —D. *Stapelia gigantea*. Pollinium wall showing thick foot layer (F) and granular layer (G), which forms separation layer between individual pollen grains. —E. *Matelela reticulata*. Inner wall separating individual pollen grains. On each side of the granular layer a foot layer (F) and intine (I) are present. —F. *Tylophora flanagani*. Pollinium wall showing columellae (C), thin foot layer (F), and intine (I). Scale bars: A–C, E & F = 1 μm ; D = 10 μm .

proximal walls are thinner. In the Secamonoideae, as represented here by *Secamone* R. Br., the distal walls have a three-layered exine structure (outer tectum, well-developed granular layer, and a foot

layer) subtended by an intine. The outer tectum can also be present as a double layer (Civeyrel, 1995). The proximal walls between tetrads are, however, reduced and only consist of a granular exine layer

subtended by an intine. In the Secamonoideae there is thus a reduction in wall layers in the inner walls, whereas in Periplocoideae the inner walls have all the layers (tectum, granular layer, and intine) and are only thinner than the distal wall. The reduction in inner wall layers in Secamonoideae was also observed by Civeyrel (1995) and Verhoeven and Venter (1994a) in *Pervillaea* Decne. The reduction of wall layers in the inner walls of Secamonoideae is similar to that observed in *Fockea* (Asclepiadoideae, Fockeeae). The reduction in wall layers from Periplocoideae to Secamonoideae supports a more advanced position of the latter at the base of the Asclepiadoideae, as has been found in various independent studies (Sennblad & Bremer, 1996; Civeyrel et al., 1998; Potgieter & Albert, 2001). It is also interesting that the subfamilies Periplocoideae and Secamonoideae as well as the tribe Fockeeae, all regarded as primitive, are restricted to the Old World. In the Periplocoideae pollen mother cell division is of the simultaneous type (Safwat, 1962) just as it is in the Apocynaceae. In *Secamone* the pollen mother cell division is also of the simultaneous type, agreeing in this respect with that of Apocynaceae s. str. and Periplocoideae (Safwat, 1962).

In the Asclepiadoideae the pollinium is covered by a pollinium wall, and single pollen grains, which lack apertures, are present. In the Asclepiadoideae, two successive meiotic divisions produce linear tetrads (Safwat, 1962). The single grains develop as linear tetrads but the cells round off and cannot be identified as tetrads (Dannenbaum & Schill, 1991). The pollinium wall, also referred to as pollinial pellicle (Swarupanandan et al., 1996), envelope (Vijayaraghavan & Shukla, 1976), or ectexine (Dannenbaum & Schill, 1991), consists of a tectum (distal exine layer), thin granular layer, foot layer, and intine. The tectum (distal exine layer), granular layer, and foot layer together form the ectexine. Individual grains are separated from each other by a thin granular layer, which is flanked, on both sides, by the foot layer and intine. *Tylophora flanaganii* in Asclepiadeae differs from the other genera investigated. Instead of a solid distal exine subtended by a granular layer, columellae are present and the foot layer is present as a very thin layer. *Tylophora* R. Br. is a heterogeneous assemblage (Liede, 1996) of about 50 species (Victor et al., 2000). Schill and Jäkel (1978) observed a wide spectrum of pollinium wall sculpturing in *Tylophora*, varying from tectate-perforate, to semitectate, to intectate. At this stage it is not clear how the pollinium structure of *Tylophora flanaganii* relates to that of the other Asclepiadoideae.

Tylophora, as well as pollinia of other genera of Asclepiadoideae, requires further studies.

The general pollinium structure of the genus *Fockea* differs from that found in other Asclepiadoideae in that the pollinium consists of tetrads and, further, it is not covered by a pollinium wall; in addition, the attachment of pollinium to corpusculum is not by a well-developed caudicle. However, the distal tetrad wall of *Fockea* shows the typical distal pollinium wall structure of Asclepiadoideae (tectum, thin granular layer, and foot layer). The pollinium in *Fockea* is very similar to that in *Secamone* of the subfamily Secamonoideae. In both the pollinium consists of tetrads and is not covered by a pollinium wall, and in both the inner walls are reduced. The pollinia in the two genera differ only in the structure of the distal wall in that the granular layer is well developed in Secamonoideae and thin in *Fockea* (Table 1). All these characters support a position for *Fockea* intermediate between Secamonoideae and the more advanced Asclepiadoideae. This position is supported by independent studies of translator ontogeny (Kunze, 1993) as well as molecular phylogeny obtained from sequences of both *matK* (Civeyrel et al., 1998) and *trnL* (Potgieter & Albert, 2001).

Nilsson (1990) studied pollen of *Holarrhena pubescens* (Buch.-Ham.) Wall. ex G. Don, pointing out the similarity between pollen grains of *Holarrhena* (Apocynaceae: Plumerioideae) and those of certain taxa of the Apocynaceae: Apocynoideae, e.g., *Alafia*, *Pleioceras*. Later *Holarrhena* and related genera (subtribe Holarrheninae) were transferred to the Apocynoideae for several reasons, including corolla lobe aestivation, differentiation of anthers, pollen morphology, relationship between gynoecium and androecium, comose seeds, and secondary chemistry, by Endress et al. (1990). The exine in *Holarrhena* and *Alafia* (also in Apocynoideae) consists of a tectum subtended by a granular stratum reminiscent of the exine structure in the Periplocoideae. In *Kopsia flavida* Blume (Rauvolfioideae) Nilsson (1990) observed an exine subdivided into two strata by a discontinuous line of osmiophilic substance. This exine structure is very similar to the pollinium wall of the Asclepiadoideae. Based on a number of phylogenetic reconstructions, these similarities must, however, be considered as parallelisms.

In the family Apocynaceae s.l., there is a phylogenetic line of development that is reflected in pollen structure. The evolutionary trends that can be followed are 3- or 4-colporate grains (Rauvolfioideae), which are more primitive than the 3-porate grains in Apocynoideae. Tetrads consisting of loosely attached grains in *Apocynum* L. s.l. (Nilsson et al., 1993) (Apocynoideae) can be regarded as

more advanced than 3-porate grains. Within the Periplocoideae the tetrads of *Raphionacme*, having numerous pores, could be regarded as more advanced than tetrads of genera with 4 to 6 pores (Nilsson et al., 1993). Pollinia (observed in 7 genera of the Periplocoideae: *Decalepis*, *Epistemma*, *Finlaysonia*, *Gymnanthera*, *Hemidesmus*, *Schlechterella*, and *Streptocaulon*) can be regarded as more advanced than single tetrads. Genera with pollinia, however, do not group in the same clade (Venter & Verhoeven, 2001). Genera with pollinia are also not necessarily more advanced in flower structure. *Hemidesmus* shows the most advanced condition in the valvate corolla lobe aestivation, anthers postgenitally fused into a ring, and the staminal tube (Nilsson et al., 1993; Endress & Bruyns, 2000). Of the Periplocoideae with pollinia, the multi-porate tetrads in the pollinium of *Schlechterella* differ significantly from the nearly inaperturate tetrads in the pollinia of all other taxa studied. This suggests that pollinia may have arisen twice within the Periplocoideae: once in Asia, and once in Africa.

If the type of pollinium and translator (which together form the pollinarium) in Asclepiadoideae are considered the most advanced, trends of development in that direction can be observed beginning already in the Apocynaceae s. str., especially *Apocynum*, in which tetrads are shed onto a simple sticky band-like translator (Nilsson et al., 1993). In the Periplocoideae the pollinia are free, consist of tetrads, and the distal walls are inaperturate or only vestigially porate. Pores are, however, present in proximal walls. Correlated with the appearance of an inaperturate distal wall is a reduction in proximal wall thickness. In the Secamonoideae, where the pollinarium has four pollinia, the pollinia are attached to a translator, which varies from consisting of only a corpusculum in *Pervillaea* (Omlor, 1996; Klackenberg, 1995a, b), to corpusculum with weakly developed caudicles in *Secamone* (Civeyrel, 1995), to corpusculum and two well-developed caudicles in *Secamonopsis* (Omlor, 1996; Civeyrel & Rowe, 2001). The pollinium in Secamonoideae consists of tetrads and is not covered with a pollinium wall. The tetrads are inaperturate and the proximal walls are reduced in thickness and wall layers. In *Fockea* (Fockeeae, Asclepiadoideae) the pollinium is similar to those in the Periplocoideae and Secamonoideae in that it consists of tetrads and is not covered with a pollinium wall. The pollinia are, however, reduced to two per pollinarium. *Fockea* also shows a similarity with the Secamonoideae (except *Secamonopsis*) in that the pollinia are not attached to distinct caudicles, but are nearly sessile

on a single dorsal plate (Kunze, 1993). The two pollinia per pollinarium are, however, characteristic for the Asclepiadoideae. The exine structure in *Fockea* is also very similar to that found in the rest of the Asclepiadoideae.

The presence of all the wall layers in proximal and inner walls, together with the tetrads coherent into a pollinium that does not become attached to a translator during ontogeny, could be interpreted as support for an independent evolution of pollinia in the Periplocoideae from that in Secamonoideae and Asclepiadoideae. It is also interesting to note that the individual grains in tetrads of *Camptocarpus* (Periplocoideae), as well as the single grains of *Kopsia flavida*, have an exine wall structure very similar to that of the individual pollen grains in the Asclepiadoideae pollinium, showing that the genetic information for this type of wall structure is also present in the more primitive groups.

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Appendix 1. Taxa examined.

Taxon	Voucher information
Periplocoideae	
<i>Baroniella camptocarpoides</i> Costantin & Gallaud	Verhoeven & Venter (1994a)
<i>Baroniella linearis</i> (Choux) Bullock	Verhoeven & Venter (1994a)
<i>Baseonema camptocarpoides</i> Choux	Verhoeven & Venter (1994a)
<i>Baseonema lineare</i> Choux	Verhoeven & Venter (1994a)
<i>Camptocarpus acuminatus</i> (Choux) Venter	Verhoeven & Venter (1994a)
[as <i>Tanulepis acuminata</i> (Choux) Choux]	
<i>Camptocarpus linearis</i> Decne.	Verhoeven & Venter (1994a)
[as <i>Tanulepis linearis</i> (Decne.) Choux]	
<i>Camptocarpus mauritianus</i> (Lam.) Decne.	Verhoeven & Venter (1994a)
[as <i>C. bojeri</i> Jum. & H. Perrier]	
<i>Camptocarpus semihastatus</i> (Decne.) Klack.	Verhoeven & Venter (1994a)
[as <i>Harpanema acuminatum</i> Decne.]	
<i>Camptocarpus sphenophyllus</i> (Baker) Venter	Verhoeven & Venter (1994a)
[as <i>Tanulepis sphenophylla</i> Balf. f. ex Baker]	
<i>Cryptolepis albicans</i> Jum. & H. Perrier	Verhoeven & Venter (1994a)
<i>Cryptolepis grandidieri</i> Roxb. ex R. Br.	Verhoeven & Venter (1994a)
<i>Cryptostegia grandiflora</i> R. Br.	Verhoeven & Venter (1994a)
<i>Cryptostegia madagascariensis</i> Bojer ex Decne.	Verhoeven & Venter (1994a)
<i>Curroria decidua</i> Planch. ex Benth.	Verhoeven & Venter (1993)
<i>Curroria macrophylla</i> A. R. Smith	Verhoeven & Venter (1993)
<i>Curroria migiurtina</i> (Chiov.) Bullock	Verhoeven & Venter (1993)
<i>Curroria volubilis</i> (Schltr.) Bullock	Verhoeven & Venter (1993)
<i>Decalepis arayalpathra</i> (J. Joseth & V. Chandras)	Verhoeven & Venter (1998a)
Venter	
<i>Decalepis hamiltonii</i> Wight & Arn.	Verhoeven & Venter (1998a)
<i>Decalepis nervosa</i> (Wight & Arn.) Venter	Verhoeven & Venter (1998a)
<i>Decalepis salicifolia</i> Bedd. ex Benth. & Hook. f.	Verhoeven & Venter (1998a)
[as <i>Utleria salicifolia</i> Bedd. ex Benth. & Hook. f.]	
<i>Finlaysonia curtisii</i> (King & Gamble) Venter	Verhoeven & Venter (1998a)
[as <i>Gongylosperma curtisii</i> King & Gamble]	
<i>Finlaysonia insularum</i> (King & Gamble) Venter	Verhoeven & Venter (1998a)
[as <i>Meladerma insularum</i> King & Gamble]	
<i>Finlaysonia khasiana</i> (Kurz) Venter	Verhoeven & Venter (1998a)
[as <i>Stelmacrypton khasianum</i> (Kurz) Baill.]	
<i>Finlaysonia maritima</i> Backer ex K. Heyne	Verhoeven & Venter (1998a)
<i>Finlaysonia obovata</i> Wall.	Verhoeven & Venter (1998a)
<i>Finlaysonia pierrei</i> (Costantin) Venter	Verhoeven & Venter (1998a)
[as <i>Atherolepis pierrei</i> Costantin]	
<i>Gonocrypta grevei</i> Baill.	Verhoeven & Venter (1994a)
<i>Gymnanthera nitida</i> R. Br.	Verhoeven & Venter (1998a)
<i>Harpanema acuminatum</i> Decne.	Verhoeven & Venter (1994a)
<i>Hemidesmus indicus</i> (L.) Schult.	Verhoeven & Venter (1998a)
<i>Ischnolepis tuberosa</i> Jum. & H. Perrier	Verhoeven & Venter (1994a)
<i>Mondia ecornuta</i> (N. E. Br.) Bullock	Verhoeven & Venter (1993)
<i>Mondia whitei</i> (Hook. f.) Skeels	Verhoeven & Venter (1993)
<i>Pentopetia androsaemifolia</i> Decne.	Verhoeven & Venter (1994a)
<i>Periploca acuminata</i> Rahman & Wilcock	Verhoeven & Venter (1994b)
<i>Periploca aphylla</i> Decne.	Verhoeven & Venter (1994b)
<i>Periploca graeca</i> L.	Verhoeven & Venter (1994b)
<i>Periploca sepium</i> Bunge	Verhoeven & Venter (1994b)
<i>Periploca visciformis</i> (Vatke) Schum.	Verhoeven & Venter (1994b)
<i>Petopentia natalensis</i> (Schltr.) Bullock	Verhoeven et al. (1989)
<i>Raphionacme abyssinica</i> Chiov.	Verhoeven & Venter (1998a)
<i>Raphionacme hirsuta</i> (E. Mey.) R.A. Dyer	Verhoeven & Venter (1988)
<i>Raphionacme madiensis</i> S. Moore	Verhoeven & Venter (1988)
<i>Raphionacme palustris</i> Venter & R. L. Verh.	Verhoeven & Venter (1988)
<i>Schlechterella africana</i> (Schltr.) K. Schum.	Verhoeven & Venter (1998b)

Appendix 1. Continued.

Taxon	Voucher information
<i>Streptocaulon baumii</i> Decne.	Verhoeven & Venter (1998a)
<i>Streptocaulon cumingii</i> (Turcz.) Vill.	Verhoeven & Venter (1998a)
<i>Streptocaulon extensum</i> Wight	Verhoeven & Venter (1998a)
<i>Streptocaulon juvenas</i> (Lour.) Merr. [as <i>S. griffithii</i> Hook. f.] [as <i>S. tomentosum</i> Wight]	Verhoeven & Venter (1998a)
<i>Streptocaulon kleinii</i> Wight & Arn.	Verhoeven & Venter (1998a)
<i>Streptocaulon sylvestre</i> Wight	Verhoeven & Venter (1998a)
<i>Tacazzea apiculata</i> Oliv.	Verhoeven et al. (1989)
<i>Tanulepis acuminata</i> Choux	Verhoeven & Venter (1994a)
<i>Tanulepis linearis</i> (Decne.) Choux	Verhoeven & Venter (1994a)
<i>Tanulepis sphenophylla</i> Balf. f.	Verhoeven & Venter (1994a)
Secamonoideae	
<i>Pervillea venenata</i> Baill. [as <i>Menabea venenata</i> Baill.]	Verhoeven & Venter (1994a)
<i>Secamone gerrardii</i> Harv. ex Benth.	SOUTH AFRICA. KwaZulu-Natal: Eshowe, 27/10/1962, <i>H. J. T. Venter</i> 645 (BLFU). SOUTH AFRICA. Northern Province: Soutpansberg, 20/10/1984, <i>N. Jacobson</i> 3409 (PRE).
Asclepiadoideae, Fockeeae	
<i>Fockea angustifolia</i> K. Schum.	SOUTH AFRICA. Mpumalanga: Marble Hall, 27/11 1972, <i>Vorster & Jackson</i> 2160 (PRE).
<i>Fockea multiflora</i> K. Schum.	NAMIBIA. Okaruwizu, 08/10/1960, <i>Giess & Wiss</i> 3310 (PRE).
<i>Fockea sinuata</i> (E. Mey.) Druce	SOUTH AFRICA. Northern Cape: Britstown, 11/03/1988, <i>L. Smook</i> 6854 (PRE).
Asclepiadoideae, Marsdenieae	
<i>Dregea macrantha</i> Klotzsch	NAMIBIA. Etosha Game Park, No date, <i>K. Tilney</i> 1131 (PRE).
<i>Gymnema sylvestre</i> (Retz.) R. Br. ex Schultes	SOUTH AFRICA. Northern Province: Messina, 08/01/1974, <i>G. K. Theron</i> 2965 (PRE).
Asclepiadoideae, Ceropegieae	
<i>Caralluma speciosa</i> N. E. Brown	TANZANIA. Lake Manyara National Park, 25/03/1968, <i>Greenway & Kanuri</i> 13244 (PRE).
<i>Ceropegia stapeliiformis</i> Haw.	SOUTH AFRICA. Eastern Cape: Jansenville, 12/1953, <i>B. Stevens</i> 4 (BLFU).
<i>Hoodia gordonii</i> (Masson) Sweet ex Decne.	NAMIBIA. Ochta, 24/10/1981, <i>H. J. T. Venter</i> 8628 (BLFU).
<i>Stapelia gigantea</i> N. E. Br.	SOUTH AFRICA. KwaZulu-Natal: Mtunzini, 10/03/1965, <i>H. J. T. Venter</i> 1830 (BLFU).
Asclepiadoideae, Asclepiadeae	
<i>Asclepias curassavica</i> L.	SOUTH AFRICA. Free State: Bloemfontein, -/05/1993, <i>R. L. Verhoeven</i> 614 (BLFU).
<i>Cynanchum ellipticum</i> (Harv.) R. A. Dyer	SOUTH AFRICA. KwaZulu-Natal: Mtubatuba, 20/05/1968, <i>H. J. T. Venter</i> 4749 (BLFU).
<i>Glossonema revoilii</i> Franch.	ETHIOPIA. Road Dagahour to Jijiga, 19/03/1941, <i>O. West</i> 5433 (PRE).
<i>Matelea brevicoronata</i> (B. L. Rob.) Woodson	U.S.A. Texas, <i>Prinzie</i> 218 (MO).
<i>M. reticulata</i> (Engelm. ex A. Gray) Woodson	U.S.A. Texas, <i>Prinzie</i> 230 (MO).
<i>Microlooma sagittatum</i> (L.) R. Br.	SOUTH AFRICA. Western Cape: Vredendal, 08/08/1977, <i>A. leRoux</i> 2155 (PRE).
<i>Pentarrhinum insipidum</i> E. Mey.	SOUTH AFRICA. Mpumalanga: Middelburg, 16/10/1968, <i>G. K. Theron</i> 1875 (PRE).
<i>Tylophora flanaganii</i> Schltr.	SOUTH AFRICA. KwaZulu-Natal: Port Shepstone, 27/06/1967, <i>R. Strey</i> 5789 (PRE).
<i>Xysmalobium undulatum</i> (L.) W. T. Aiton	SOUTH AFRICA. Gauteng: Randburg, 01/11/1976, <i>L. Liebenberg</i> 8528 (PRE).

PHYLOGENETIC
RELATIONSHIPS OF
SECAMONOIDEAE BASED ON
THE PLASTID GENE *matK*,
MORPHOLOGY, AND
BIOMECHANICS¹

Laure Civeyrel² and Nick Rowe³

ABSTRACT

Relationships between Secamonoideae and their closest relatives in Apocynaceae, as well as genera and species within the subfamily, have been examined, using a molecular phylogeny with the plastid gene *matK*. Our molecular results show that Secamonoideae form a monophyletic group. Delimitations of genera are also discussed together with the evolution of pollinarium and biomechanical characters within species. The patterns of pollinial organization, as well as change in growth forms, are mapped onto a phylogenetic tree obtained from the plastid gene *matK*, all of which together significantly contribute toward a better understanding of the evolution of the Apocynaceae s.l.

Key words: Apocynaceae, biomechanics, *matK*, palynology, phylogeny, pollinia, Secamonoideae.

The Secamonoideae (Endlicher, 1838) are the smallest subfamily of the former Asclepiadaceae *sensu stricto* with only 7 widely accepted genera and less than 200 species. It is the sister group of the Asclepiadoideae, which are by far the largest subfamily of the Apocynaceae s.l. There is currently little phylogenetic information about Secamonoideae (Safwat, 1962; Nicholas & Baijnath, 1994; Civeyrel, 1996; Klackenberg, 1996b; Omlor, 1996; Sennblad & Bremer, 1996; Klackenberg, 1997; Civeyrel et al., 1998). This paper uses additional data to examine relationships between Secamonoideae and their closest relatives, and focuses on the relationships between genera and species within the subfamily itself. In addition, the study presents observations on pollinarium characters as well as biomechanical changes in growth form within the group.

The systematic position of Secamonoideae based on morphological characters is complicated because some characters appear to link them more

closely to basal Apocynaceae s. str. and Periplocoideae than to Asclepiadoideae (Omlor, 1996). Many characters have been proposed for delimitation of the Secamonoideae, but they frequently occur outside the subfamily as well. For example, Secamonoideae have simultaneous microsporogenesis (Safwat, 1962) together with pollen in tetrads, which are rectangular or T-shaped and similar to the few basal Apocynaceae with pollen in tetrads, as well as the tetrads found in Periplocoideae (Nilsson et al., 1993; Verhoeven & Venter, 1993, 1994, 1998, 2001 this volume). In contrast, apomorphic Asclepiadoideae have successive microsporogenesis and linear tetrads (Safwat, 1962; Puri & Shiam, 1966; Bruyns & Forster, 1991). Nevertheless, pollen in rectangular tetrads has also been described in the genus *Fockea* (Asclepiadoideae) (Civeyrel, 1996), although details concerning microsporogenesis for this genus are lacking. As in all Apocynaceae (Endress et al., 1983), a structurally defined compitum has been found in Secamonoideae. A

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